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(54) Title: PROCESS FOR REDUCING RNA CONCENTRATION IN A MIXTURE OF BIOLOGICAL MATERIAL USING DIATOMACEOUS EARTH

(57) Abstract

The invention relates to a process for reducing RNA concentration in a mixture of biological material, comprising the steps of: (a) providing a mixture of biological material having a first concentration of RNA; (b) filtering the mixture through a diatomaceous earth material to produce a filtrate having a second concentration of RNA, wherein the second concentration is less than the first concentration; and (c) collecting the filtrate having a reduced RNA concentration. In one aspect, the diatomaceous earth material serves to purify recombinant plasmid DNA from an RNA component in a mixture of biological material, and in another aspect, it serves to separate different forms of soluble RNA in a mixture of biological material.

PROCESS FOR REDUCING RNA CONCENTRATION IN A MIXTURE OF BIOLOGICAL MATERIAL USING DIATOMACEOUS EARTH

FIELD OF THE INVENTION

The invention relates to a process for reducing RNA concentration in a mixture of biological material using diatomaceous earth.

BACKGROUND OF THE INVENTION

Molecular biology depends for its importance on the existence of purified recombinant DNA plasmids. *E.g.*, Thompson, BioChromatography 1:68 (1986); Current Protocols in Molecular Biology, Greene Publishing Assoc. & Wiley, 1987; and Sambrook, Fritsch, and Maniatis, Molecular Cloning, A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, 1989. Of critical significance to the development of a practical technology was the discovery that cells could incorporate extrachromosomal genetic material. Consequently, independently-replicating extrachromosomal DNA, such as plasmid DNA, can be used as a vehicle for the insertion into and amplification of any given DNA segment, from a wide variety of biological sources, in a suitable cell host which will maintain this genetic material.

Advances have been made in the fermentation and tissue culture processes used for both analytical and preparative scale growth of prokaryotic and eukaryotic cells recombinant plasmid DNA. These biological processes of synthesis generate the plasmid DNA product together with a complex mixture of cellular components including lipids, carbohydrates, lipoproteins, proteins, polysaccharides, chromosomal DNA, ribosomes, RNA and other macromolecular components. It is necessary to isolate plasmid DNA in a pure form for subsequent application.

Numerous procedures have been developed independently for the purification of cellular plasmid DNA. However, all of these processes share three basic features: (1) cellular growth; (2) cellular lysis; and (3) separation of plasmid DNA from cellular RNA and DNA. Various extractions are of key importance for the accurate, reproducible purification of plasmid DNA. These extractions are employed to remove the bulk of contaminating molecules (*e.g.*, proteins, cellular DNA, RNA, etc.), especially when applied to complex biological systems. Proper extractions are essential for both chromatography and non-chromatography processes of plasmid purification.

A step-by-step conventional protocol for purification of plasmid DNA from l liter (1-2 g wet weight) of E. coli cells is presented in Table 1.

TABLE 1: PURIFICATION OF PLASMID DNA FROM 1 LITER (1-2 G) OF E. COLI CELLS

CELL LYSIS:

1. Resuspend cells in a final volume of 7.0 ml with Buffer A. Buffer A is 25 mM Tris-HCl (pH 8.0), 50 mM Na_2EDTA, 1% (w/v) glucose.
2. Add 14.0 ml of Buffer B; gently mix by inversion; incubate 10 min on ice. Buffer B is 0.2 N NaOH, 1% SDS.

SUMMARY OF THE INVENTION

The invention provides a process for reducing RNA concentration in a mixture of biological material, comprising the steps of: (a) providing a mixture of biological material having a first concentration of RNA; (b) filtering the mixture through a diatomaceous earth material to produce a filtrate having a second concentration of RNA, wherein the second concentration is less than the first concentration; and (c) collecting the filtrate having a reduced RNA concentration.

According to the invention, the RNA concentration may be reduced by at least about 10%, or advantageously by at least about 25%, or favorably by at least about 50% or preferrably by at least about 85%.

Also, according to the invention, the mixture of biological material may be a cell lysate.

In one embodiment, the diatomaceous earth material is composed of about 90% SiO_2. It may be calcined. It may be flux calcined (as opposed to straight calcined). It may be acid washed (to analytical grade quality).

In another embodiment, the diatomaceous earth material has a dry density of about 10 lbs/cu.ft. It may possess a median particle size of about 22.3 microns. It may possess a median cake pore size of about 7 microns.

In a preferred embodiment, the diatomaceous earth material is composed of about 89.6% SiO_2, about 4.0% Al_2O_2, about 1.5% Fe_2O_3, about 0.2% P_2O_5, about 0.2% TiO_2, about 0.5% CaO, about 0.6% MgO, and about 3.3% $Na_2O + K_2O$.

In another aspect, the invention provides a process for reducing RNA concentration in a mixture of biological material, comprising the steps of: (a) providing a mixture of biological material having a first concentration of RNA; (b) exposing the mixture to a diatomaceous earth material to produce a product which, following separation from the diatomaceous earth material by filtration, centrifugation or sedimentation, has a second concentration of RNA, wherein the second concentration is less than the first; and (c) collecting the product having a reduced RNA concentration.

In still another aspect, the invention provides a process for purifying recombinant plasmid DNA from a RNA component in a mixture of biological material, comprising the steps of: (a) providing a mixture of biological material comprising recombinant plasmid DNA and having a first concentration of RNA; (b) filtering the mixture through a diatomaceous earth material to produce a filtrate comprising the recombinant plasmid DNA and having a second concentration of RNA, wherein the second concentration is less than the first concentration; and (c) collecting the filtrate having a reduced RNA component.

The recombinant plasmid DNA may be a nucleic acid-based pharmaceutical.

In yet another aspect, the invention provides a process for separating different forms of soluble RNA in a mixture of biological material, comprising the steps of: (a) providing a mixture of biological material having a first and a second form of soluble RNA; (b) filtering the mixture through a diatomaceous earth material to produce a filtrate comprising the first form of soluble RNA and a product comprising the second

Crude diatomaceous earth material is mined and then processed to serve as a filter aid. It is usually processed by milling, calcining and air classification to give a finished, virtually inert filter aid which is predominantly silica. When deposited on a filter membrane, the diatomaceous earth material forms a rigid but porous filter cake which sieves out the particulate matter in liquid as it passes through the filter.

Filtration using diatomaceous earth material is commonly a two-step operation. First, a thin protective layer of filter aid, called the precoat, is built up on the filter medium by recirculating a filter aid slurry. After precoating, filter aid is added to the liquid to be filtered. As filtering progresses, the filter aid, mixed with the suspended solids from the unfiltered liquid, is deposited on the precoat. Thus, a new filtering surface is continuously formed. The minute filter aid particles provide countless microscopic channels which entrap suspended impurities but allow clear liquid to pass through without clogging.

An efficient, economic filter aid will have rigid, intricately shaped, porous, individual particles; will form a highly permeable, stable, incompressible filter cake; will remove even the finest solids at high rates of flow; and will be chemically inert and essentially insoluble in the liquid being filtered. Diatomaceous earth material meets these requirements due to the wide variety of intricately shaped particles and inert composition.

Filter aid grades are available providing a wide range of particle sizes to meet industrial filtration requirements. Diatomaceous earth material that is the finest will usually give the highest clarity and lowest flow rate. To create this grade, diatomite may be selectively quarried, dried, milled and air-classified. To make coarser, faster flow rate filter aids, diatomite is calcined and air-classified. These are called straight calcined grades. To obtain still larger particles, a flux is added before calcination giving the flux-calcined filter aids. These are the coarsest grades.

In addition to these standard grades of diatomaceous earth material, specialized products may be produced for a wide variety of filtration applications. Acid-washed filter aids are available by acid washing to reduce iron and calcium content for use where exceptional purity is needed. Analytical grade filter aids are obtainable by maximum acid washing for use as an aid in analytical work or where extra exceptional purity is desired.

Selection of the proper filter aid grade is a compromise between high clarity and low flow rate. The best filter aid is the grade that provides the fastest flow rate while maintaining an acceptable degree of clarity, which must be determined and specified by the filter aid user. Selection is empirical and within the skill of workers in the art.

For a given filtration, clarity of filtrate is governed principally by: grade and amount of filter aid for filtration; grade and amount of filter aid for precoat; length of cycle; and filtration rate.

Determination of the degree of clarity obtainable by any one grade of filter aid can be gained by running filtration tests using proper techniques, for example, on a Buchner funnel, and is within the level of skill in the art.

The invention is useful to purify recombinant plasmid DNA from a RNA component in a mixture of biological material. In this embodiment, one provides a mixture of biological material comprising recombinant plasmid DNA and having a first concentration of RNA. One then filters the mixture through a diatomaceous earth material to produce a filtrate comprising the recombinant plasmid DNA and having a second concentration of RNA, where the second concentration is less than the first concentration. Finally, one collects the filtrate having a reduced RNA component.

This process is useful for the purification of a nucleic acid-based pharmaceutical.

The process of the invention is not limited to circular, supercoiled plasmids (*e.g.*, supercoiled monomers, dimers, concatemers), but applies generally to other supercoiled DNA molecules including chloroplasts, mitochondria, etc., and to different forms (*e.g.*, different replicating forms) of circular DNA molecules, and also to linear DNA molecules, *e.g.*, chromosomal DNA and oligonucleotide DNA.

Neither is the invention limited to plasmids having a particular size or constitution. It has general applicability across plasmids of every kind, regardless of size and constitution. *See* Examples. We have used the invention to purify, *e.g.*, pBR322- and pUC- based, bi-cistronic and mono-cistronic, and human- and nonhuman- originating, plasmids alike.

Additionally, this process may be applicable to the separation of different forms of RNA in a mixture of biological material. There is an effort in some laboratories to perform gene therapy with RNA rather than DNA. A purification process that selectively removes host contaminant RNA has tremendous value, when the object of the separation is the recovery of a particular form of RNA.

In this embodiment, one provides a mixture of biological material having a first and second form of soluble RNA. One then filters the mixture through a diatomaceous earth material to produce a filtrate comprising the first form of RNA and a product comprising the second form of RNA that collects in the diatomaceous earth material. Finally, one collects the filtrate. Alternatively, one may elute the second form of RNA from the diatomaceous earth material, given that is the preferred form.

A preferred protocol for purification of recombinant plasmid DNA from a RNA component in a mixture of biological material is presented in Table 2. Here the mixture is a cell lysate. Also, this is a start-to-finish protocol where plasmid DNA is purified to pharmaceutical-grade standards for use as a nucleic acid-based pharmaceutical.

TABLE 2: PROCESS FOR PURIFICATION OF PHARMACEUTICAL-GRADE PLASMID DNA

1. Cell Paste
2. Resuspend Cells in Buffer
3. Lyse Cells in Dilute Base and Detergent
4. Acidify Lysate to Precipitate Host DNA and E. coli Proteins
5. Remove Cell Debris and Other Impurities by Centrifugation, Filtration or Sedimentation
6. Clarify Supernatant by Filtration with a Diatomaceous Earth Material
7. Precipitate DNA with PEG-8000 from Clarified Filtrate

as flow rate, clarity, grade and amount of filter aid for filtration, grade and amount of filter aid for precoat, length of cycle, filtration rate, throughput, and permeability, etc., will very much depend on the reduction in the concentration of RNA to be achieved. An approximation of the degree of reduction can be gained by running conventional filtration tests. The amount of RNA in the filtrate can be determined in a number of ways, for example, by routine biochemical analyses. *See* Example 4.

DNA Precipitation. Polyethylene glycol (PEG, *e.g.*, PEG-8000) is added to the filtrate to 5-15% (w/v), plus NaCl to 0.3-1.5 M. The PEG suspension is stirred preferably overnight at 2-8°C. The DNA precipitate is collected by adding approximately 25 g/l of diatomaceous earth material to the PEG suspension and filtering through a (preferably precoated) filter membrane arranged in a table top Buchner funnel. The DNA precipitate is captured in the cake and recovered by suspending the cake in TE buffer (0.01 M Tris-base pH 8.0 + 0.001 M EDTA).

RNA, Protein and Lipopolysaccharide Removal. Ammonium acetate is added to the TE buffer to 2.5 M and stirred for approximately 30 minutes at 2-8°C. The suspension, which still contains diatomaceous earth material, is filtered through a (preferably precoated) filter membrane arranged in a table top Buchner funnel. The DNA filtrate is then optionally clarified by sub-micron filtration.

Final DNA Precipitation. A final DNA precipitation is performed with 0.6 volumes of cold isopropanol for a minimum of 2 hours at -20°C. The precipitated DNA is centrifuged in a Sorvall table top centrifuge for 30 minutes at 2000 x g or equivalent. The DNA pellets are resuspended in column buffer prior to gel filtration chromatography.

Gel Filtration Chromatography. A Pharmacia S-1000 tandem size exclusion column, DNA exclusion limit of 20,000 bp, (Pharmacia, Piscataway, NJ) is poured. The S-1000 matrix is an inert and highly stable matrix that is prepared by covalently cross-linking allyl dextran with N,N'-methylenebisacrylamide. The column is poured in two Pharmacia XK26/100 columns (Pharmacia, Piscataway, NJ) with a final bed height of 80-85 cm (2.6x80cm) resulting in a total column volume of approximately 900 ml and a total length of approximately 160 cm. The columns are individually pressure packed in one direction, reversed and connected in series for equilibration and operation. The column is equilibrated in column buffer and run at an appropriate flow rate. Cleared lysate plasmid DNA is filtered through a 0.2 μm syringe filter and loaded onto the column. Column operation and fractionation are automated with a Pharmacia FPLC (Pharmacia, Piscataway, NJ). Fractions (approximately 0.5 - 5% of column volume) are collected over the product elution zone and analyzed by 0.8% agarose gel electrophoresis. The exact range of product elution is determined from gel analysis. Appropriate fractions are pooled and precipitated with 2 volumes of cold ethanol. This column purified DNA is stored at -20°C until needed for preparation of standard bulk plasmid DNA. Following chromatography, the column and FPLC are sanitized with at least one column volume of 0.2 M NaOH.

Standard Bulk Plasmid DNA Preparation. The ethanol precipitated, column purified DNA is spun at maximum speed in a Sorvall table top centrifuge for 30 minutes at 4-10°C or equivalent. The pellets are air-dried and pooled. The pooled pellets are resuspended in injection vehicle. The DNA is then filtered

The pHLA-B7 plasmid was about 4900 bp in size. It was a pBR322-based plasmid containing a bacterial origin of replication. It encoded the heavy (human HLA-B7 cDNA) and light (chimpanzee B-2 Microglobulin cDNA) chains of a Class 1 MHC antigen designated HLA-B7. These two proteins were expressed on a bi-cistronic mRNA. Eukaryotic cell expression of this mRNA was dependant on a Rous Sarcoma Virus promoter sequence derived from the 3' Long Terminal Repeat. Expression was also dependent on a transcription termination/polyadenylation signal sequence derived from the bovine growth hormone gene. Expression of the heavy chain was regulated by the 5' cap-dependent protein translation start site. Expression of the light chain was regulated by a Cap Independent Translational Enhancer (CITE) sequence derived from the Encephalomyocarditis Virus. The plasmid also encoded a kanamycin resistance gene derived from Tn903.

In this purification, 500 g of wet cell paste was processed, and 29.1 mg of purified plasmid DNA was recovered.

Cell Lysis. About 500 g of cell paste was resuspended in 3 liters of cold Solution I (61 mM glucose + 25 mM Tris buffer pH 8.0 + 10 mM EDTA at 5°C) with stirring at room temperature. Next, 6 liters of Solution II (0.2 N NaOH + 1% SDS) was added and mixed end-over-end until homogeneous. This was incubated in wet ice for 10 minutes. Then, 4.5 liters of cold Solution III (3.0 M potassium acetate pH 5.0 at 5°C) was added, mixed end-over-end until a white flocculent precipitant formed, and incubated in wet ice for 10 minutes.

Filtration. Approximately 1200 grams of Celite® diatomaceous earth was added to the lysate and mixed by swirling until homogeneous. A table top Buchner funnel was assembled using Whatman # 113 filter paper. The filter paper was pre-coated with Celite® diatomaceous earth in deionized H_2O. The lysate was then poured through the filter paper in the filter assembly to permit a Celite® cake to build up on the filter paper. The total time to filter 13.5 liters was 15 minutes. The total filtrate recovered was 12 liters.

DNA Precipitation. PEG-8000 was added to the filtrate to 10% (w/v), plus NaCl to 0.58 g per gram of original cell mass. The PEG suspension was stirred overnight at approximately 4°C. The DNA precipitate was collected as follows. About 325 grams of Celite® diatomaceous earth was added to the PEG suspension and mixed by swirling until homogeneous. A table top Buchner funnel was assembled using Whatman # 113 filter paper. The filter paper was pre-coated with Celite® diatomaceous earth in deionized H_2O. The PEG suspension was then poured through the filter paper in the filter assembly to permit a Celite® cake to build up on the filter paper. The cake was aspirated dry. The DNA precipitate was captured in the Celite® cake and recovered by suspending the cake in 1 liter of TE buffer (0.01 M Tris-base pH 8.0 + 0.001 M EDTA). The total volume came to 1700 ml.

RNA, Protein and Lipopolysaccharide Removal. Ammonium acetate was added to the TE buffer to 2.5 M for 1800 ml and stirred for about 1 hour at approximately 4°C. A table top Buchner funnel was assembled using Whatman # 113 filter paper. The filter paper was pre-coated with Celite® diatomaceous earth in deionized H_2O. The ammonium acetate suspension, which still contained diatomaceous earth, was

TABLE 3: QUALITY CONTROL CRITERIA

TEST	SPECIFICATION	METHOD
Size Identity	Approximates: 4900 bp	Agarose Gel Electrophoresis
Restriction Sites	Approximates predicted: XhoI/XbaI - 3500 & 1400 bp, BglII/XhoI - 2100, 1700 & 1000 bp	Agarose Gel Electrophoresis
Circular Plasmid DNA	> 95% of visualized nucleic acid	Agarose Gel Electrophoresis
A260/A280 Ratio	1.75 to 2.00	UV Absorbance
E coli DNA	< 0.01 μg/μg plasmid DNA	Southern Slot Blot
RNA	Non-visualized on gel	Agarose Gel Electrophoresis
Protein	Undetectable	BCA Colorimetric Assay
Pyrogenicity	Not pyrogenic at 5 μg/Kg rabbit body weight	Rabbit Pyrogen Assay
Endotoxin	< 0.1 EU/μg plasmid DNA	Limulus Amebocyte Lysate (LAL) Assay
Sterility	No growth through 14 days	Fluid Thioglycollate Assay
Potency	50-200% of reference	*In Vitro* Transfection/ Fluorescence
General Safety Test	Passes	per 21 C.F.R. 610.11

EXAMPLE 3. POTENCY OF PURIFIED pHLA-B7 PLASMID

Potency of pHLA-B7 plasmid purified according to Example 1 was determined by HLA-B7 gene expression in HALL cells (a human melanoma cell line) following lipid-mediated *in vitro* transfection using DMRIE/DOPE.

From 200,00 to 400,000 HALL cells were seeded per well into a 6-well plate the day before transfection. Cells were a >75% confluent monolayer prior to transfection. The cells were transfected with 5 μg plasmid DNA in the presence of 5 μg DMRIE (synthesized in house) and 5 μg DOPE (synthesized by Avanti Polar Lipids, Inc., Alabaster, AL). The cells were incubated at 37°C, 5% CO_2 throughout. A reduced serum medium, *e.g.*, Opti-MEM (GIBCO BRL Life Technologies, Baltimore, MD), supplemented with fetal calf serum, was added to the cells 1-4 hours and 24 hours post-transfection. Cells were harvested 48 hours post-transfection.

HLA-B7 expression on the cell surface was measured by labelling with anti-HLA-B7 mouse antibody, followed by a fluorescent secondary antibody (anti-mouse IgG monoclonal antibody R-phycoerrythin conjugate).

Cell Lysis. The cell paste was resuspended in 6 ml per gram wet bacterial weight of cold Solution I (61 mM glucose + 25 mM Tris buffer pH 8.0 + 10 mM EDTA at 5°C) with stirring at room temperature. Next, 12 ml per gram wet bacterial weight Solution II (0.2 N NaOH + 1% SDS) was added and mixed end-over-end until homogeneous. This was incubated in wet ice for 10 minutes. Then, 9 ml per gram wet bacterial weight of cold Solution III (3.0 M potassium acetate pH 5.0 at 5°C) was added, mixed end-over-end until a white flocculent precipitant formed, and incubated in wet ice for 5 minutes.

Filtration. The cell debris was removed from the lysate by centrifugation. The supernatant was collected and clarified by adding approximately 25g/l Celite® diatomaceous earth and filtering through a table top Buchner funnel. Alternatively, approximately 90 g/l Celite® diatomaceous earth could be added directly to the lysis solution and mixed by swirling until homogeneous. The lysate could then be filtered through a table top Buchner funnel.

DNA Precipitation. PEG-8000 was added to the filtrate to 10% (w/v), plus NaCl to 0.58 g per gram of original cell mass. The PEG suspension was stirred overnight at approximately 4°C. The DNA precipitate was collected by adding about 25 g/l of Celite® diatomaceous earth to the PEG suspension and filtering through a table top Buchner funnel. The DNA precipitate was captured in the Celite® cake and recovered by suspending the cake in TE buffer (0.01 M Tris-base pH 8.0 + 0.001 M EDTA).

RNA, Protein and Lipopolysaccharide Removal. Ammonium acetate was added to the TE buffer to 2.5 M and stirred for about 30 minutes at approximately 4°C. The suspension, which still contained Celite® diatomaceous earth, was filtered through a table top Buchner funnel. The DNA filtrate was then clarified by sub-micron filtration.

Final DNA Precipitation. A final DNA precipitation was performed with 0.6 volumes of cold 2-propanol for a minimum of 2 hours at -20°C. The precipitated DNA was centrifuged in a Sorvall table top centrifuge for 30 minutes at 2000 x g. The DNA pellets were resuspended in physiological saline prior to gel filtration chromatography.

Gel Filtration Chromatography. A Pharmacia S-1000 tandem size exclusion column, DNA exclusion limit of 20,000 bp, (Pharmacia, Piscataway, NJ) was poured in two Pharmacia XK26/100 columns (Pharmacia, Piscataway, NJ) with a final bed height of 80-85 cm (2.6x80cm) resulting in a total column volume of approximately 900 ml and a total length of approximately 160 cm. The columns were individually pressure packed in one direction, reversed and connected in series for equilibration and operation. The column was equilibrated in physiological saline and run at a flow rate of approximately 0.75 ml/min. Cleared lysate plasmid DNA was filtered through a 0.2 μm syringe filter and loaded onto the column. Column operation and fractionation were automated with a Pharmacia FPLC (Pharmacia, Piscataway, NJ). Fractions (approximately 0.5 - 5% of column volume) were collected over the product elution zone and analyzed by 0.8% agarose gel electrophoresis. The exact range of product elution was determined from gel analysis. Appropriate fractions were pooled and precipitated with 2 volumes of cold ethanol. This column purified DNA

with 2.5 μg plasmid DNA in the presence of 0.5 μg DMRIE (synthesized in house) and 0.5 μg DOPE (synthesized by Avanti Polar Lipids, Inc., Alabaster, AL). The cells were incubated at 37°C, 5% CO_2 throughout. A reduced serum medium, *e.g.*, Opti-MEM (GIBCO BRL Life Technologies, Baltimore, MD), supplemented with fetal calf serum, was added to the cells 1-4 hours and 24 hours post-transfection. Cell supernatant was harvested 80 hours post-transfection.

IL-2 expression in the cell supernatant was measured by an enzyme amplified sensitivity immunoassay (Medgenix ELISA, Medgenix Diagnostics, Fleurus, Belgium). Potency was 50-200% relative to a reference lot.

EXAMPLE 8. PURIFICATION OF pCMVBGH PLASMID

The pCMVBGH plasmid was purified. This plasmid was about 4300 bp in size. It contained all the elements found in pCMVIL2BGH, excluding the human IL-2 coding sequence.

Using the purification steps described in Example 6 above, 503 g of wet cell paste was processed, and 14.1 mg of purified plasmid DNA was recovered.

We provide a process for reducing the RNA concentration in a mixture of biological material by using a diatomaceous earth material. This is a new use for a diatomaceous earth material. We appreciate that the diatomaceous earth material retains as much as about 85% of the available RNA.

While particular embodiments of the invention have been described in detail, it will be apparent to those skilled in the art that these embodiments are exemplary rather than limiting, and the true scope of the invention is that defined within the attached claims.

16. A process for purifying recombinant plasmid DNA from a RNA component in a mixture of biological material, comprising the steps of:

(a) providing a mixture of biological material comprising recombinant plasmid DNA and having a first concentration of RNA;

(b) filtering said mixture through a diatomaceous earth material to produce a filtrate comprising said recombinant plasmid DNA and having a second concentration of RNA, wherein the second concentration is less than the first concentration; and

(c) collecting said filtrate having a reduced RNA component.

17. The process of Claim 16, wherein said recombinant plasmid DNA is a nucleic acid-based pharmaceutical.

18. A process for separating different forms of soluble RNA in a mixture of biological material, comprising the steps of:

(a) providing a mixture of biological material having a first and a second form of soluble RNA;

(b) filtering said mixture through a diatomaceous earth material to produce a filtrate comprising said first form of soluble RNA and a product comprising said second form of soluble RNA that collects in said diatomaceous earth material; and

(c) collecting said filtrate, or eluting said second form of RNA from said diatomaceous earth material.

19. The process of Claim 18, wherein one of said forms of RNA is a nucleic acid-based pharmaceutical.

20. A process for purifying recombinant plasmid DNA from a RNA component in a mixture of biological material, comprising the steps of:

(a) providing a mixture of biological material comprising recombinant plasmid DNA and having a first concentration of RNA;

(b) adding a diatomaceous earth material to said mixture;

(c) mixing the result of step (b) to form a suspension;

(d) pouring said suspension through a diatomaceous earth precoated filter membrane to allow a diatomaceous earth cake to collect on said filter membrane and to form a filtrate comprising said recombinant plasmid DNA and having a second concentration of RNA, wherein the second concentration is less than the first concentration; and

(e) collecting said filtrate having a reduced RNA component.

16. A process for purifying recombinant plasmid DNA from a RNA component in a mixture of RNA and DNA, comprising the steps of:

(a) providing a solution comprising recombinant plasmid DNA and RNA, said solution having a first concentration of RNA;

(b) filtering said solution through an effective amount of diatomaceous earth, wherein the RNA will bind to diatomaceous earth more strongly than will DNA, to produce a filtrate comprising said recombinant plasmid DNA and having a second concentration of RNA, wherein the second concentration is less than the first concentration; and

(c) collecting said filtrate having a reduced RNA component, with the proviso that said process is conducted in the absence of a chaotropic agent.

17. The process of Claim 16, wherein said recombinant plasmid DNA is a component of a pharmaceutical preparation.

18. A process for separating different forms of soluble RNA in a solution of RNA, comprising the steps of:

(a) providing a solution of RNA, said solution having a first and a second form of soluble RNA; and

(b) filtering said solution through an effective amount of diatomaceous earth, wherein some forms of RNA will bind to diatomaceous earth more strongly than will other forms of RNA, to produce a filtrate comprising said first form of soluble RNA and a product comprising said second form of soluble RNA that collects in said diatomaceous earth, with the proviso that said process is conducted in the absence of a chaotropic agent.

19. The process of Claim 18, wherein one of said forms of RNA is a component of a pharmaceutical preparation.

20. A process for purifying recombinant plasmid DNA from a RNA component in a mixed solution of RNA and DNA, comprising the steps of:

(a) providing a solution comprising recombinant plasmid DNA and RNA, said solution having a first concentration of RNA;

(b) adding an effective amount of diatomaceous earth, wherein the RNA will bind to diatomaceous earth more strongly than will DNA, to said solution;

(c) mixing the result of step (b) to form a suspension;

(d) pouring said suspension through a diatomaceous earth precoated filter membrane to allow a diatomaceous earth cake to collect on said filter membrane and to form a filtrate comprising said recombinant plasmid DNA and having a second concentration of RNA, wherein the second concentration is less than the first concentration; and

(e) collecting said filtrate having a reduced RNA component, with the proviso that said process is conducted in the absence of a chaotropic agent.

INTERNATIONAL SEARCH REPORT

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP,A,0 555 798 (BECTON DICKSON & COMPANY) 18 August 1993 see the whole document	16,17,20
X	EP,A,0 389 063 (AKZO N,V,) 26 September 1990 see page 3 see page 9, line 2 - line 3 see page 16 section F and page 17 see page 11; example A3 see page 14; examples B5-B7 see page 19 section I	1,6
Y		16-20
Y	AGRIC. BIOL. CHEM., vol. 44, no. 8, 1980, pages 1821-1827, XP002001948 KUNINAKA ET AL.: "Extraction of RNA from yeast packed into column without isomerization" see the whole document	1,6,15
Y	CHEMICAL ABSTRACTS, vol. 88, no. 25, 19 June 1978 Columbus, Ohio, US; abstract no. 188151u, page 556; column 188149; XP002001950 see abstract & JP,A,78 012 900 (YAMASA SHOYU CO) 4 February 1978	1,6,15
Y	NUCLEIC ACIDS RESEARCH, vol. 21, no. 4, 1993, page 1044 XP002001949 CARTER ET AL.: "An inexpessive and simple method for DNA purifications on silica particles" see the whole document	18,19
Y	CHEMICAL ABSTRACTS, vol. 120, no. 25, 20 June 1994 Columbus, Ohio, US; abstract no. 321505k, page 731; column 321517; XP002001951 see abstract & FUZHOU DAXUE XUEBAO, ZIRAN KEXUEBAN, vol. 20, no. 4, 1992, pages 110-111, GUUZHONG W. ET AL.: "Obtaining the high quality ribonucleic acid from yeast"	1,20

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INTERNATIONAL SEARCH REPORT

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